Mechanical properties of cytoplasm investigated by cytomagnetometry accompanied by immunofluorescent microscopy

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1 Introduction

Cytomagnetometry has been shown to be effective in probing the mechanisms of intracellular motion and rheological properties of the cytoplasm[1]-[7]. Fig. 1 shows its principle. Magnetic particles such as Fe_3O_4 are introduced into cells. Cultured cell line J774.1 are usually used for our experiments because of their phagocitic activity. The particles then exist within the organelles called phagosomes. A strong magnetic field is applied to magnetize the particles and then the phagosomes act like minute magnets. A weak magnetic field originating from the aligned magnetic dipoles is measured from the cells. We call this field “cell field”.

The cell field decays with a typical half time of 1-2 min. This magnetic relaxation is due to the randomization of the direction of the magnetic moments of phagosomes caused by their mechanical motions. Therefore, the relaxation reflects the mechanism of intracellular motion and the rheological properties of the cytoplasm. Manipulation of the phagosomes from outside can be done by applying a weak magnetic field to obtain various kinds of information about the energy of the randomizing force and the stiffness and integrity of the cytoskeleton.

In [7], we proposed a mechanical model of the tissue surrounding the phagosome to analyse the experimental results. This model was intended to be used for “twisting experiments” where phagosomes are twisted and their viscoelastic recoil is measured by cytomagnetometry. The model includes 4 parameters. The viscoelastic recoil measurement allows us to estimate all the four parameter values including E_r, the energy responsible for relaxation. On the otherhand, relaxation measurement has the advantage that it will not affect (in principle) the cytoskeletal strucures. However, it alone does not reflect the value of E_r. In this report we use the method introduced in [1,6] to estimate E_r and relaxation measurement to estimate the rest of the parameters.

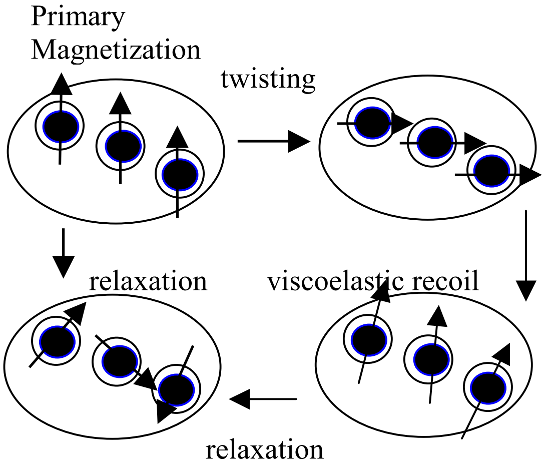

Figure 1: *Principle of cytomagnetometry.*

We also present in this report some images of J774.1 cells obtained by laser scanning microscopy under the influence of cytochalasine-B(CytB) and colchicine(Col). CytB is known to disrupt microfilaments and Col to inhibit the formation of microtubules. These drugs were used in cytomagnetometry to investigate the roles of the filamentous structures in intracellular movements of phagosomes. The optical investigations should help to interprete

2 Methods

J774.1 cells (Human Science Center, Osaka, Japan) phagocytized magnetic particles (Fe_3O_4, diameter 1.9 μm, 8 μg/0.5 million cells) and formed a monocellular layer in bottles (diameter 12 mm). The particles were magnetized by a pulse magnetic field of about 0.3T. The cell field was measured by a flux-gate magnetometer (Forster, Germany). The sensitivity of the the measuring system was in the order of 0.1 μG. For relaxation measurement, the cell field was simply measured for 6-7 minutes. For E_r measurement, a weak field was applied for 3 minutes after some relaxation took place. This magnetization does not change the magnetization of the particles themselves but it mechanically rotates the phagosomes. The weak field H(in the order of

0.1mT) gives rise to potential energy μH where μ is the magnetization of the particle. The value $a=\mu H/E_r$ determines the value of the equilibrium magnetic field through the Langevin function

$$L(a) = \coth a - 1/a \qquad (1)$$

The equilibrium field was estimated by obtaining an upper bound and a lower bound for the equilibrium value. Fig. 2 is a schema showing the method. We call this experiment "L- measurement" for simplicity.

The elastic component becomes most apparent in the viscoelastic measurement, in which T_1 s after pulse magnetization, the phagosomes are twisted by a medium field ($\cong$1mT) perpendicular to the pulse field. After T_2 (10-20)s of twisting, the field is removed and the cell field is measured which shows some recoil of the field due to mechanical elasticity of the tissues surrounding the phagosome. It is one of the purposes of this paper to compare the behavior of the parameter values (relaxation and recoil).

The cells were treated either with CytB or Col to investigate the role of microfilaments and of microtubules in causing the random rotational movements of the phagosomes and also in acting as viscoelastic elements to the phagosome motion.

For immunoflurescent microscopy of micro filaments, the cells were fixed with formaldehyde and stained with phalloidin-FITC and embedded in anti-fade kit(Molecular Probe). For visualizing microtubules, cells were fixed with glutaraldehyde and first treated with mouse anti-β-tubulin and then with anti-mouse-IgG FITC-conjugate. The cells were treated either with CytB or Col to see the effects of these drugs on the cytoskeletons. The specimen was observed under a scanning laser microscope(BioRad MRC600).

3 Model

Fig. 3 shows the 1-dimensional model used to analyze the result of the cytomagnetometric experiments. Here we give only the outline of the model and its analysis. The model consists of two viscous elements and one elastic element. The differential equations governing the model are:

$$\frac{d\theta_1}{dt} = \frac{c}{k_2}\left(\theta_1(t) - \underline{\theta}_1\right) + \frac{1}{k_2}\left(\frac{dW(t)}{dt} + f(t,\theta)\right)$$

$$\frac{d\theta_2}{dt} = \frac{1}{k_1}\left(\frac{dW(t)}{dt} + f(t,\theta)\right) \qquad (2)$$

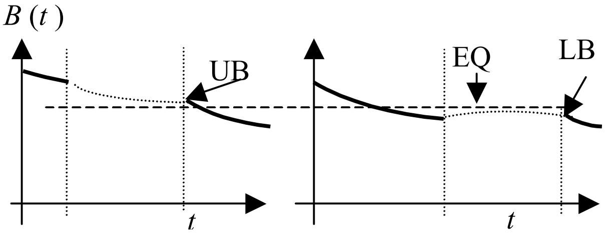

Figure 2: *The procedure to obtain an upper bound (UB) and a lower bound (LB) for the equilibrium cell field(EQ) under the influence of both the randomizing force and the external medium field.*

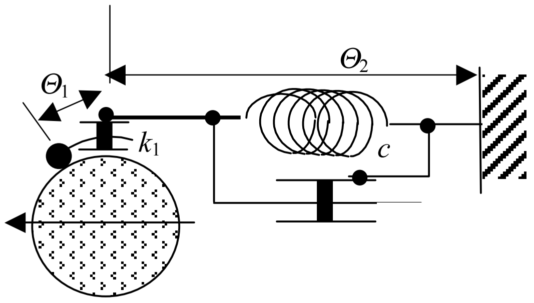

Figure 3: *The model of the mechanical properties of the tissues surrounding the phagosome for cytomag-netometry.*

where θ_1 and θ_2 are defined such that $\theta = \theta_1 + \theta_2$ is the direction of the magnetic moment of the phagosome measured from that of the measurement. $\underline{\theta}_1$ is the natural length of the elastic body for which it is in the resting state. $f(t, \theta)$ is the torque [Nm] exerted by the external field designed to achieve a particular purpose in each experiment. c comes from elasticity and has the dimension [Nm]. $W(t)$ is the Brownian motion caused by the intracellular movements such as cyto-plasmic streaming. All the parameter values are normalized with respect to the energy E_r of ranmozation caused by $W(t)$. During the relaxation, the cell field decays accoring to

$$B(t) = \exp\left(-\frac{V[\theta(t)]}{2}\right) \qquad (3)$$

where $V[\theta(t)]$ is the variance of $\theta(t)$ at time t. After pulse magnetization, the variance is given by

$$V[\theta(t)] = \alpha\left(\frac{1-e^{-\sigma t}}{\sigma k_2^2} + \frac{2(1-e^{-\sigma t})}{\sigma k_1 k_2} + \frac{t}{k_1^2}\right) \qquad (4)$$

For the twisting experiment, eq (2) was solved by Runge Kutta method for N sample processes of Brownian motion $W(t)$ and

$$\frac{1}{N}\sum_{i=1}^{N}\cos\theta(t,i)$$

was calculated where $\theta(t,i)$ is the azimuth of the i-th moment at time t calculated from the differential equation. N was 2000 to 4000 in most cases.

In the viscoelastic measurement, the measurement curve is divided into 3 distinctive parts; relaxation part, twisting part and recoiling part. We tried to fit all three parts of the curve with a single set of parameter values, which was often difficult[6].

4 Results

Fig. 4 shows an example of cell field plot after pulse magnetization showing relaxation. The data points are approximated by the curve determined by eqs.(3)(4) with parameter values normalized with respect to E_r;

$$k_1/E_r = 330,\ k_2/E_r = 475,\ c/E_r = 11$$

Fig. 5 shows an example of cell field plot for E_r estimation by L-measurement. The weak field H =0.02mT(=0.2G) and the magnetic moment $\mu = 7.11\times10^{-14}$ Am^2 of each of the particles used give $\mu H = 1.42\times10^{-18}$ Nm. One can get a =5.17 for the Langevin function (1). Then one gets $E_r = 2.75\times10^{-19}$ J. Finally we get

$$k_1 = 1.377\times10^{-16}, k_2 = 1.767\times10^{-16}, c = 1.15\times10^{-18}$$

The units are [Nms] for k_i's and [Nm] for c. Fig. 6 is the summary of the parameter changes caused by the presence of colchicine. The parameter values are plotted against the concentration of colchicine. It is seen that as time elapses after application of colchicine, k_2 and c increases while the other parameters stay rather constant. Fig. 7 shows the effects of CytB. It is seen that k_2, c and E_r decrease as the concentration becomes higher while k_1 stays quite constant.

Fig. 8 is an example of a viscoelastic recoil data fitted by the simulation curve (average of 10000 sample processes) with parameter values

$$k_1 = 7.44\times10^{-16}, k_2 = 7.77\times10^{-16}, c = 8.62\times10^{-18}$$

and $Er = 2.16\times10^{-18}$ J.

Fig.9 shows immunofluorescent images of microfilaments. Microfilaments are prominent in

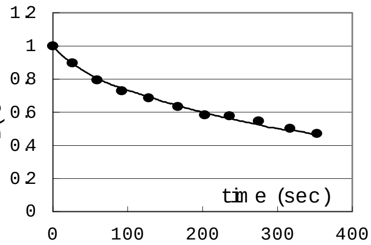

Figure 4: *A relaxation curve fitted with a model curve.*

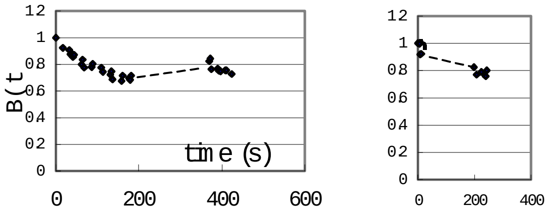

Figure 5: *"L-measurement" to estimate the equilibrium cell field under a weak external field.* Upper (right) and lower (left) bounds are obtained.

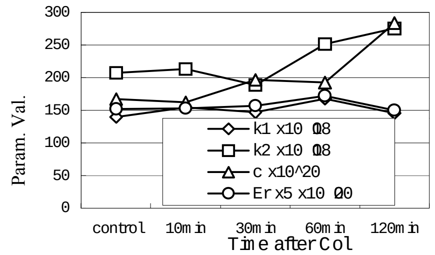

Figure 6: *Effects of colchicine on the parameter values estimated by relaxation and L-measurements*

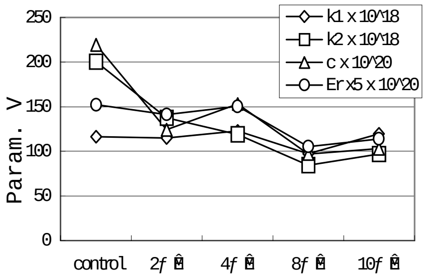

Figure 7: *Effects of cytochalasin B on the parameter values estimated by relaxation and L-measurements.*

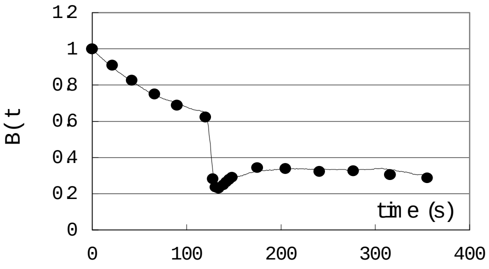

Figure 8: *An example of visco-elastic recoil data fitted by a simulation curve.*

peripheral area of the cells and treatment with CytB considerably reduces the amount of microfilaments. Fig. 10 shows again the cells stained for microfilaments. One of the cells contains a magnetic particle(the same kind used in cytomag-netometry). The cross section along the line was scanned and reconstructed. Fig. 11 shows the effect of colchicine on microtubules.

5 Discussion and Conclusion

It was shown that by relaxation measurement together with E_r measurement one can estimate the model parmaeters and that the behavior of the parameter values is different from that shown by viscoelastic measurement[7]. This does not necessarily imply inadequacy of the model, Rather, we expect that estimation of parameter values in one model by different experimental methods should reveal the behavior of the mechanical properties in different situations since they may well show much nonlinear behavior. The immunofluorescent microscopy was used to observe the situations to which cytoskeletal filaments are exposed during cytomagnetometry using CytB and Colch. Quantitative analysis of the optical results will further help to understand the cytomagnetometric results.

References

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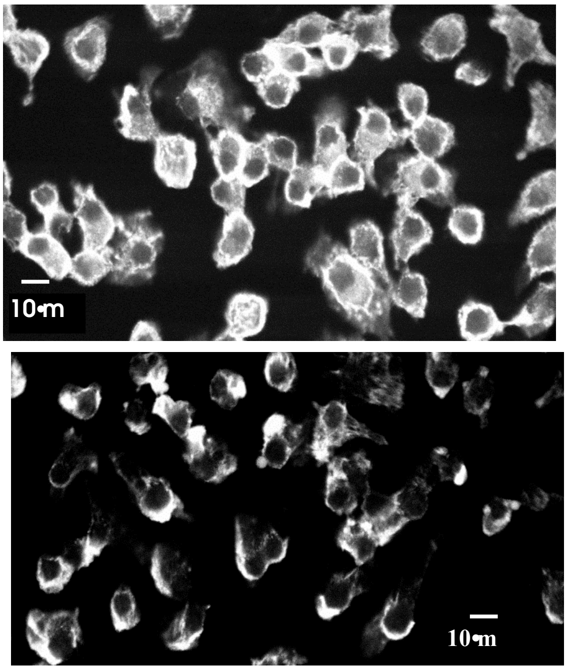

Figure 9: *Immunofluorescent images of micro-filaments by laser scanning microscope. upper:control; (b):with CytB(10 μM/l).*

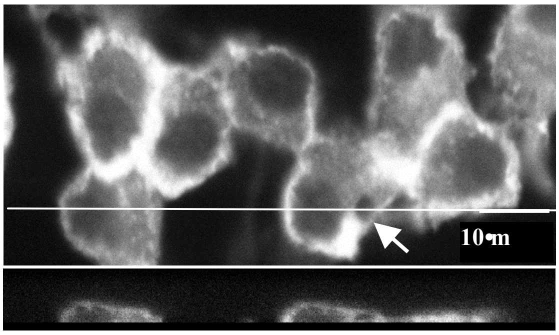

Figure 10: *Cells imaged for microfilaments(a). One cell has a magnetic particle (arrow) where vertical scanning was performed along the white line and reconstructed in the lower panel.*

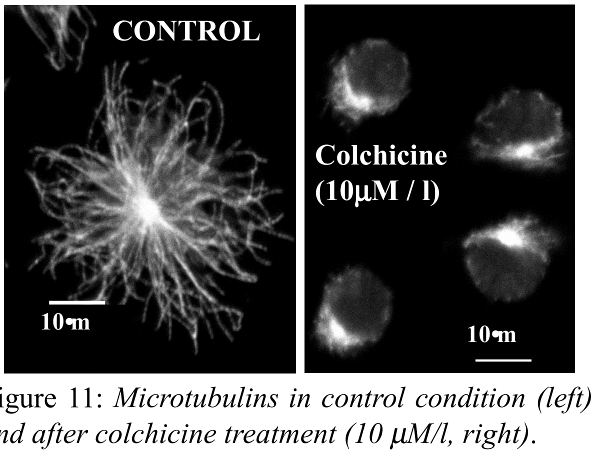

Figure 11: *Microtubulins in control condition (left) and after colchicine treatment (10 μM/l, right).*